

Fig. 6 shows the structure of the alpha amylase as in Fig. 1 wherein differences in Loop 8 are emphasized.

*B1*  
*Chattel*  
*(SEQ ID NO:13)*  
Fig. 7 shows differences in the alpha amylase structure in the loop between strand 3 and helix 3 of domain A.

*one*  
*C2*  
*sub*  
*H1*  
Fig. 8 depicts an alpha amylase from WO95/26397.

A map of plasmid pDN1528 is shown in Fig. 9.

A map of plasmid pJeEN1 is shown in Fig. 10.--

**IN THE CLAIMS:**

Please cancel claims 1-69 and add new claims 70-111 as follows:

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--70. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to one or more of, Q298, G299, G301, Y302,

L307, N309, Q340, F343, F403, H405, H406, D407, , G410, L427, I428, D430, G433, K436, N473, G474 and G475 in *Bacillus licheniformis*.

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Canty

--71. The alpha-amylase according to claim 70, wherein said alpha-amylase is produced by *Bacillus*.

--72. The alpha-amylase according to claim 71, wherein said alpha-amylase is produced by *Bacillus licheniformis*, *Bacillus amyloliquefaciens* or *Bacillus stearothermophilus*.

--73. A detergent comprising the alpha-amylase according to claim 70.

--74. A starch liquefaction composition comprising the alpha-amylase according to claim 70.

--75. The alpha-amylase according to claim 70, wherein said alpha-amylase further comprises a substitution or deletion at one or more residues equivalent to M15, V128, H133, W138, N188, A209 and/or M197 in *Bacillus licheniformis*.

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--76. The alpha-amylase according to claim 70 which is modified by substituting an amino acid residue at a position corresponding to one or more of G301, H405, H406 and/or K436 in *Bacillus licheniformis*.

*BJ*  
*Con*  
*Sub*  
*F6*  
--77. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to Q298 in *Bacillus licheniformis*.

--78. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to G299 in *Bacillus licheniformis*.

--79. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein

Docket No. 0776/1F216-US2

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said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to G301 in Bacillus licheniformis.

--80. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to Y302 in Bacillus licheniformis.

--81. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to L307 in Bacillus licheniformis.

--82. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein

said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to N309 in *Bacillus licheniformis*.

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Cathy

--83. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to Q340 in *Bacillus licheniformis*.

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--84. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to F343 in *Bacillus licheniformis*.

--85. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein

said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to F403 in Bacillus licheniformis.

--86. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to H405 in Bacillus licheniformis.

--87. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to H406 in Bacillus licheniformis.

--88. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein

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said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to D407 in *Bacillus licheniformis*.

BZ  
Constr

--89. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to G410 in *Bacillus licheniformis*.

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--90. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to L427 in *Bacillus licheniformis*.

--91. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein

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Cristy

said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to I428 in *Bacillus licheniformis*.

--92. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to D430 in *Bacillus licheniformis*.

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--93. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to G438 in *Bacillus licheniformis*.

--94. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein



said alpha -amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to K436 in Bacillus licheniformis.

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--95. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to N473 in Bacillus licheniformis.

--96. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to G474 in Bacillus licheniformis.

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Fg  
--97. An alpha-amylase comprising an A domain, a C domain and a calcium binding site, wherein said calcium binding site is associated with said A domain and said C domain comprises ligand residues in said A domain and/or said C domain, wherein

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said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to G475 in *Bacillus licheniformis*.

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conty

--98. The alpha-amylase of claim 70 wherein said alpha-amylase is modified to alter the characteristics of said calcium binding site and thereby alter the performance of said alpha-amylase by substituting an amino acid residue at a position corresponding to one or more of, Q298, G299, G301, Y302, L307, F343, H405, H406, D407, I428, D430, and G475 in *Bacillus licheniformis*.

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--99. The alpha-amylase of claim 98 wherein said alpha-amylase further comprises a substitution or deletion at one or more residues equivalent to A209 and/or M197 in *Bacillus licheniformis*.

--100. The alpha -amylase according to claim 98 which is modified by substituting an amino acid residue at a position corresponding to one or more of G301, H405, and/or H406 in *Bacillus licheniformis*.

--101. The alpha-amylase according to claim 98, wherein said alpha-amylase is produced by *Bacillus*.

--102. The alpha-amylase according to claim 98, wherein said alpha-amylase is produced by *Bacillus licheniformis*, *Bacillus amyloliquefaciens* or *Bacillus stearothermophilus*.

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--103. A detergent comprising the alpha-amylase according to claim 98.

--104. A starch liquefaction composition comprising the alpha-amylase according to claim 98.

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--105. An alpha-amylase variant of an alpha-amylase having the sequence SEQ. ID # 2 wherein at least one amino acid residue present in a fragment corresponding to amino acid residues 322-346 of SEQ. ID # 2 has been substituted with another residue present in a fragment corresponding to amino acid residues 291-313 of SEQ. ID # 10.

--106. An alpha-amylase wherein at least one amino acid residue present in a fragment corresponding to amino acid residues 322-346 of SEQ. ID # 2 has been substituted with another residue thereby resulting in modified calcium binding properties of said amylase.

B2  
amity

--107. An alpha-amylase variant wherein at least one amino acid residue within a distance of 10 Angstrom or less from a calcium ion binding site of said alpha-amylase has been replaced by another amino acid residue thereby modifying the calcium dependence and/or affinity for calcium ion of said amylase.

--108. The alpha-amylase of claim 107 wherein said at least one amino acid residue is selected from the group consisting of V102, I103, N104, H105, K106, R125, W155, W157, Y158, H159, F160, D161, G162, T163, Y175, K176, F177, G178, K180, A181, W182, D183, W184, E185, V186, S187, N192, Y193, D194, Y195, L196, M197, Y198, A199, D200, I201, D202, Y203, D204, H205, P206, V208, A209, D231, A232, V233, K234, H235, I236, K237, F238, F240, L241, A294, A295, S296, T297, Q298, G299, G300, G301, Y302, D303, M304, R305, K306, L307, W342, F343, L346, Q393, Y394, Y396, H405, H406, D407, I408, V409, R413, E414, G415, D416, S417, V419, A420, N421, S422, G423, L424, I428, T429, D430, G431, P432, V440, G441, R442, Q443, N444, A445, G446, E447, T448, W449, I462, G475, Y480, V481, Q482, R483.

--109. The alpha-amylase of claim 108 wherein said at least one amino acid residue that has been replaced is at least two amino acid residues.